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REACTIONS OF DENERVATED VOLUNTARY MUSCLE, AND THEIR BEARING ON THE MODE OF ACTION OF PARASYMPATHETIC AND RELATED NERVES.

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I. Introductory.

A paper recently published by one of us with H. W. Dudley [Dale and Dudley, 1929] described the isolation and identification of acetylcholine from the perfectly fresh spleens of certain animals. The occasion of the first chemical recognition of this substance, as a natural constituent of the body, was taken to review the evidence suggesting its connection with certain normal effects of parasympathetic nerves, and also with the contractures produced in denervated voluntary muscles by stimulating parasympathetic and other nerves having, normally, only vaso-dilator actions. The possibility of explaining this association, between the normal vaso-dilator actions and the effects on motor denervated muscles, by attributing both to the peripheral liberation of a substance having both types of action, had presented itself to several who have worked on these phenomena. It was mentioned by Bremer and Rylant [1924] and again by Hinsey and Gasser [1928]. The view published from this laboratory differed from these only in suggesting acetylcholine, which produces both types of effect with unique intensity, as most likely to be the substance in question. The recent careful review by Gasser [1930] of work on these contracture responses of denervated muscles, from their first discovery by Philippeaux and Vulpian [1863] to the present day, makes it unnecessary for us to give a connected historical survey; we need only refer, in their proper places, to earlier observations bearing on our own.

Gasser, in his review, mentions some difficulties in the way of accepting acetylcholine as the common mediator of these effects, in particular the well-known ease with which the vaso-dilator effect of acetylcholine is obliterated by small doses of atropine, which leave the otherwise similar vaso-dilator effects of the chorda tympani and of

antidromic stimulation of sensory nerves [Reid Hunt, 1918] practically unaffected.

Adrenaline, again, has a potent antagonism to the production of contracture in denervated voluntary muscle by intravascular injection of acetylcholine; on the other hand, it does not always diminish, but often even increases the otherwise similar contracture produced by stimulation of the appropriate, normally vaso-dilator nerve. It is our purpose in this paper to record certain observations on the manner of production of these contractures in denervated muscle by stimulation of such nerves, and on the depression or augmentation by other substances of these effects, whether produced by nerve stimulation or by acetylcholine and analogous substances. Our object has been to examine more critically the evidence for a chemical intermediary in the nerve effects, and for its suggested identification as acetylcholine.

II. Methods.

For experiments on denervated voluntary muscles with their natural vascular supply we have used the musculature of the tongue and the gastrocnemius. All these experiments have been made on cats and dogs, those using the gastrocnemius being on cats, those on the tongue mostly on dogs, with a few on cats. All the records of movements of the tongue reproduced in the figures were obtained in experiments on dogs. Two experiments were made on muscles artificially perfused with blood, and for these the dog's gastrocnemius was used. For experiments on muscle isolated from the body slips of diaphragm were found to be suitable; these were obtained from young cats, in which the phrenic nerve was easily reached in the neck for division. A few experiments on rats showed that a sensitive preparation could be obtained from them also.

Denervation. The preliminary nerve sections were all done under anæsthesia with ether, and with full asepsis. For the denervation of the tongue the hypoglossal nerve was isolated in its course medial to the submaxillary gland, and about 1 cm. was excised. For experiments on the response of the denervated gastrocnemius to drugs only, the sciatic was cut high in the thigh, or the tibial nerve in the popliteal space; the former operation being used for most of the experiments on cats, the latter for those on dogs. In experiments made on the response of the gastrocnemius to antidromic stimulation of sensory fibres, or on the effect of sympathetic nerve stimulation on its response to drugs, the spinal roots were cut intradurally, so that the ventral root fibres degenerated and those in the dorsal roots remained. Section of the fifth

to ninth post thoracic roots caused degeneration of all motor fibres to the gastrocnemius, so that the Sherrington contracture was produced on peripheral stimulation of the sciatic. In three cases a part of one ventral root was left intact, as detected by the production of a weak tetanus on faradization of the uncut sciatic nerve in the spinal preparation. Since nearly the whole of the gastrocnemius in these cases had lost its motor nerve fibres, it responded with good contractures to acetylcholine, and was used for testing the antagonism to this effect of stimulating the abdominal sympathetic chain, the sciatic nerve being left intact. Denervation of the half-diaphragm was carried out by avulsion of the right phrenic nerve, so as to eliminate the possibility of fibres being left intact from an accessory root, not easily reached and identified from the neck incision. The nerve was seized below the junction of the second main root from the fifth cervical nerve, and was pulled out of the thorax. Dissection after death showed that it had been broken in the neighbourhood of the right auricle. After the preliminary nerve sections 10 to 24 days were allowed for degeneration in different experiments.

For the experiments on the reactions of denervated muscle with natural circulation, at the end of the degeneration period, the animal was either anæsthetized fully with chloralose following ether, or was made into a spinal preparation by high section of the spinal cord and destruction of the brain under ether. The former method was used for all the experiments on the tongue, the latter for most of those on the gastrocnemius. In the earlier observations on the tongue, including both those made on the cat, we used only visual observation, supplemented in one case by photography. Later we found it more convenient to make a mechanical record of the movements, using a method similar to that employed by v. Rijnberk [1915]. The animal lay on its back, with the head on the table, kept in position by a cord passing behind the upper canine teeth. The lower jaw was held in the widely open position by a similar cord attached to a support. Transmission to the tongue of respiratory movements of the larynx were minimized by passing a pointed steel rod through the soft parts just caudal to the hyoid bone, and fixing its two ends to vertical supports at the sides of the table. The tongue fell naturally into the roof of the mouth, and a fine thread, sewn through the tip, connected it to the end of one arm of a light lever, placed almost vertically above it. The lever moved in a plane radial to the recording drum, on which its other end recorded by a frontal writing point. A small weight attached to the lever kept the

thread taut and the tip of the tongue just raised from the roof of the mouth.

A contracture of the denervated half causes the whole tongue to rise from the roof of the mouth, and such movement was recorded by a fall of the writing point of the lever. It will be clear, however, that such a tracing does not record the contraction of any particular muscle system in the tongue. It gives only a crude record of the movement of the tip of the tongue, due to the rise of the whole organ with the contracture; but this was sufficient for our purpose.

In experiments on the cat's gastrocnemius the femur was held rigidly by a steel rod transfixing its lower end, the muscle was dissected clear of others, and the Achilles tendon was connected to an isometric spring lever, as in Dale and Gasser's experiments [1926]. To record the movements of the isometric lever on the same drum as the blood-pressure, etc., we attached its end to the same simple lever, with frontal writing point, as was used for recording the tongue contractures. Downward movements of the curve, accordingly, give a practically quantitative record of rise of tension in the gastrocnemius. The exposed muscle was kept warm and moist by a stream of Ringer's solution, passing through a coil in a thermostat bath and directed in a jet on to the upper side of the muscle, which was covered with a thin wisp of cotton wool. In perfusion experiments on the dog's gastrocnemius the muscle was left in its natural relations, the tendon only being isolated, detached with a portion of the calcaneum, which was sawn across, and then connected to the tension lever. For isolated strips of diaphragm the ordinary thermostat bath, designed for isolated strips of involuntary muscle, was used, a vigorous stream of oxygen bubbles being maintained through the Ringer's solution. A light isotonic lever, giving about a six to seven-fold magnification, was used for recording the contractures.

Nerve stimulations were made through platinum electrodes. These were usually fixed in a clamp, the nerve being laid across them and protected from drying, so that successive stimulations were applied to the same stretch of nerve. Induction shocks were used, obtained from the secondary of a cored coil, the current in the primary, obtained from a single accumulator cell, being in most cases interrupted by the ordinary automatic spring hammer. For special purposes Lewis's rotating interruptor was used to give break-shocks at different known rates. The coil and primary current used, with automatic interruption, gave a stimulus just perceptible on the tip of the observer's tongue with the secondary coil at 16 c.c. distance.

For intravenous injection of drugs a cannula was tied in the external jugular or femoral vein. For intra-arterial injections cannulæ were tied into suitable branches, which were clamped except at the moment of injection, so that the drug was carried straight to the denervated muscle. For the tongue the arterial injection cannula was usually tied into the central stump of the superior thyroid artery, so that injection was made into the blood flowing in the carotid artery. For experiments on the denervated gastrocnemius, both internal iliac arteries and the external iliac of the normal side were tied. The central stump of one (or sometimes two) of these tied arteries was provided with an injection cannula, so that drugs were injected into the aorta at the bifurcation and carried by the blood directly to the denervated leg.

III. Results.

(1) *Development and fatigue of response to nerve stimulation.*

The general features of the response are most easily studied in the tongue, though there is nothing to suggest that the contracture of the motor-denervated leg muscles, with stimulation of the antidromic vaso-dilator fibres, is not wholly analogous. In the latter case the fibres concerned have been traced to endings on the blood vessels, and not on the muscle fibres [Hinsey, 1927] and have been shown to be the fibres responsible for vaso-dilatation [Hinsey and Gasser, 1930]. It is reasonable to assume, then, that in the tongue also, in spite of the endings of small nerve fibres on the muscle described by Boeke [1921], the contracture is due to stimulation of the vaso-dilator fibres, as Heidenhain [1883], indeed, long ago concluded. Heidenhain, however, also recognized that it was not a direct effect of increased blood flow, since it persisted after stoppage of the circulation and could even be obtained in a tongue excised with the chorda-lingual nerve attached. The general nature of the contracture forbids its attribution to stimulation of muscle fibres by action currents in these small nerve fibres [cf. v. Rijnberk, 1915; Gasser, 1930], and Heidenhain, recognizing the need of some process between vaso-dilator action and stimulation of the sensitized muscle fibres, supposed that the latter had, by denervation, been rendered in some way sensitive to lymph secretion. With our present knowledge of substances which always cause arterial dilatation, and cause contracture of voluntary muscle only when motor denervated, Heidenhain's facts are more easily interpreted in terms of the liberation of such a substance. That lymph formation as such is

not the effective stimulus to contracture is easily shown. A substance like histamine is highly effective in causing lymph formation, but its injection into the circulation of the denervated muscle causes no contracture.

Heidenhain observed the dog's tongue under conditions similar to those in our experiments, except that he recorded only the beginning of its rise from the resting position, by the breaking of an electrical contact. This enabled him, however, to observe that, of a series of slow induction shocks applied to the chorda-lingual nerve, the first few seemed to be ineffective; then would follow a series of contractions, increasing in duration until they fused, so that the contact remained broken until after the shocks were stopped. He found, further, that when the rate of stimulation was increased, the latent period was not merely shortened; a smaller number of the more rapidly occurring shocks was needed to produce a recognizable contracture, than with the slower rate.

We also varied the rate of chorda-lingual stimulation, using break-shocks obtained with Lewis's rotating key, and recording the whole course of the tongue movement. Fig. 1 shows a typical set of results.

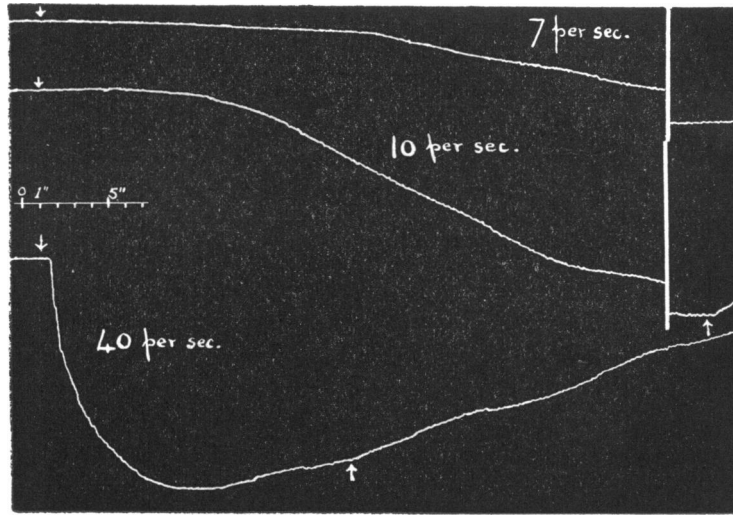


Fig. 1. Record of movement of denervated tongue of dog. Stimulation of chorda-lingual with break-shocks at different rates. Stimulation begins at ↓, ends at ↑.

The coil distance was first determined, at which, with rapid repetition of shocks, the tongue would pass into maximal contracture during

20 seconds or less of chorda-lingual stimulation. Different rates of stimulation at this constant strength of shock were then tested, an interval of 3 to 5 minutes being allowed for complete recovery after each period of stimulation.

It was found that reduction of the rate from 60 down to about 20 per sec. made no perceptible difference to the result. When the rate was reduced below 20 per sec. the effect rapidly changed. At 4 per sec. no effect could be detected. At 7 per sec. (see Fig. 1) there was a prolonged latent period; it is uncertain, indeed, whether the slight and very gradual fall of the curve, which is seen till nearly 20 seconds have elapsed from the beginning of stimulation at the arrow ↓, is due to slight contracture or merely to turgescence from vaso-dilatation. After this the curve begins to fall at a greater rate, though still slowly, and after a further 35 to 40 seconds (including a portion cut out of the record and corresponding to 20 seconds) the contracture is seen to have reached a steady low maximum, the stimulation being continued in this case beyond the end of the portion shown. At 10 per sec. (Fig. 1) the latency is shortened and the contracture progresses more rapidly when it appears. When the stimulation is stopped at the second arrow ↑, 1 minute after its commencement, the contracture has again reached a practically steady level, much higher than the maximum attained at 7 per sec., and slow relaxation begins within a second of the end of stimulation. When the stimulation is raised to 20 per sec. or more (Fig. 1 shows a response to 40 per sec.), the effect presents a very different picture. The contracture begins rapidly after a latent period of about 0·5 second, and attains its maximum in about 8 seconds; at 10 seconds from the commencement, and with continued stimulation, relaxation begins to set in; and when, at the end of about 18 seconds, the stimulation is stopped (↑), the rate of relaxation is hardly increased beyond that already attained during the later part of the stimulation. It should be noted that the small steps, just visible on the steeply falling first part of this curve, do not represent individual contractions, having no relation to the rate of stimulation; they correspond to heart beats, the tongue, with dilated arterioles, pulsating visibly. Other slight changes in the level of the curve, at wider intervals, are due to transmitted traces of movements of the larynx with respiration.

Such relations of the effect, to the rates at which shocks of constant strength are applied to the nerve, would not be easily explained on any theory of direct conduction of the nerve impulses to the muscle fibres, even if an anatomical connection were known to exist. On the other

hand they, together with all the observations made on these phenomena by earlier workers, become easy to interpret, if we suppose the direct effect of the nerve impulses to be the liberation, at their peripheral endings in relation to the blood vessels, of a labile, diffusible substance, which stimulates contracture in the sensitized muscle. In order to stimulate the muscle fibres, such a substance must be produced at a rate sufficiently in excess of the rate of its destruction, or its removal by the circulation, to reach them in an effective concentration. If stimuli of a given strength are too widely spaced, it is easy to understand that the substance liberated in response to each will be destroyed or removed before the succeeding one occurs, and that, with increasing rates of stimulation, rise of concentration to the threshold will occur with fewer stimuli and increase beyond it will be accelerated. On the other hand, a single induction shock, of sufficient strength to stimulate every fibre of the nerve, might in a suitable preparation cause the sudden liberation of sufficient substance to enable it just to reach the muscle fibres in sufficient concentration to evoke a small contracture. Such an effect of a single strong shock has been recorded by several observers, being, according to Bremer and Rylant, more commonly obtained in the tongue of the cat than of the dog.

The commencing subsidence of the contracture, during continued stimulation with shocks at 20 or more per sec., is not always seen. In certain preparations we obtained contractures which continued to increase during stimulation of the nerve for a minute or more, and even for some time after the stimulation was stopped, before beginning slowly to subside; but the type of effect seen in Fig. 1, with stimulation at 40 per sec., is commoner. Fig. 2 shows a similar effect with stimulation at 20 per sec., recorded on a slower drum. Here we have evidence of fatigue, and the question arises whether it is a fatigue of the mechanism producing the stimulant substance, or fatigue of the muscle fibres to its stimulant action. It is not due to local fatigue of the nerve to the strong electrical stimulation; shifting the electrodes to a more peripheral stretch of the nerve produces no revival of the effect. The choice lies, therefore, between temporary exhaustion of the peripheral mechanism for liberating the stimulant, and a refractoriness of the muscle to its action. Such a refractory condition is known to be produced by the various substances which evoke this type of contracture of denervated muscle on injection, including acetylcholine [cf. Gasser and Dale, 1926]. Refractoriness to the naturally liberated stimulant cannot, however, play a large part in the phenomenon here under consideration. When the

contracture is subsiding from its maximum with continued nerve stimulation, an intravascular injection of acetylcholine is still effective. After many successive stimulations of the nerves, it sometimes happens that the effect on the tongue muscle is permanently weakened, so that each further stimulus causes only a weak contracture, which rapidly subsides during the stimulation. If, during such ineffective stimulation, a small dose of acetylcholine is given by arterial injection, it produces an effect as great as that of an equal dose given without stimulation of the nerve; the effect may be even somewhat increased, as in Fig. 3, probably

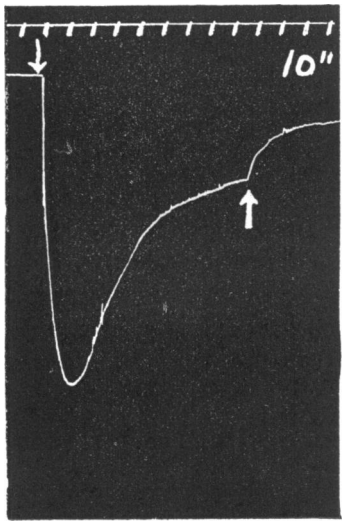


Fig. 2.

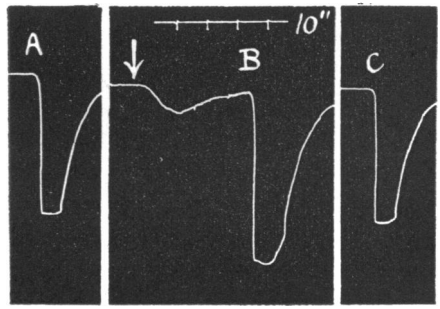


Fig. 3.

Fig. 2. Denervated tongue. Slower record. Stimulation of chorda-lingual for 90 seconds. Fatigue effect.

Fig. 3. Denervated tongue. A, B and C arterial injections of 1γ acetylcholine. Stimulation of chorda-lingual for 2 minutes from ↓.

because the already dilated vessels of the tongue enable it to reach its point of action more quickly. On the other hand, a larger injection of acetylcholine, sufficient to produce an after-depression on the effect of another similar injection, depresses also the effect of nerve stimulation. This, however, has little significance, in view of Gasser and Dale's observation that acetylcholine caused a temporary depression even of the response of the denervated muscle to direct electrical stimulation. It seems clear, in any case, that the fatigue of the response to nerve stimulation is due mainly to a weakened efficacy of the nerve impulses in

liberating the stimulant substance, at such a rate that it reaches the muscle fibres in effective concentration.

Mention should be made of other experiments in which, instead of stimulating the nerve with shocks of constant strength at different rates, we kept the rate supramaximal by use of the automatic spring hammer, and varied the strength by adjusting the distance of the secondary coil. The change of strength did not obviously affect the latency or the initial rate of development of the contracture, but its maximum extent declined rapidly with decrease of the strength of the shocks. The effect of diminishing the strength of stimulus was presumably to diminish the number of nerve fibres stimulated, and, as the results seemed, on that basis, to be compatible with almost any theory of the mechanism, we did not pursue the point.

(2) *Action of Adrenaline.*

(*a*) *Nervous contractures.*

Concerning the immediate effect of circulating adrenaline, on the contracture of denervated muscle evoked by intravascular injection of acetylcholine, or of any similarly acting drug, there is no doubt or difference of opinion. The presence of adrenaline in the circulation when such an injection is made abolishes its effect or reduces it to a very small remnant [cf. Frank, Nothmann and Hirsch-Kauffmann, 1922, 1923; Gasser and Dale, 1926]. On the other hand, while Frank and his co-workers observed that an intravenous injection of adrenaline abolished, in like manner, the Vulpian phenomenon in the dog's tongue, no reduction of this effect, under apparently similar conditions, was observed by Langworthy [1924] or by Plattner and Reisch [1926] in the cat, or by v. Rijnberk [1915] or by Orbeli and Fiedelholz [1928] in the dog. Hinsey and Gasser, however, found that an intravenous dose of adrenaline caused a temporary suppression of the Sherrington phenomenon in the cat's gastrocnemius. There was at least not a regular concordance between the recorded actions of adrenaline on the drug effects and on those of nerve stimulation. The discrepancy, it should be noted, would present difficulty for a theory attributing these latter to the liberation of any substance of the class producing the contracture, and not only for that concerned with acetylcholine.

Our first experiments on this point were made on the motor-denervated tongues of cats and dogs, with direct, visual observation of the effects. Their result was to confirm the statements of Langworthy, Plattner, v. Rijnberk and Orbeli, that adrenaline does not abolish

or perceptibly diminish the response of the tongue to chorda-lingual stimulation. We observed, on the contrary, that adrenaline caused a pronounced prolongation of the response to a brief period of stimulation. Normally the tongue began its relaxation from complete contracture as soon as the stimulation was stopped, and had returned to its original position in a time only about twice as long as that taken in attaining the maximum of contracture. A stimulus given shortly after an intravenous injection of adrenaline caused maximal contracture at a rate apparently normal; when the stimulus ceased, however, the contracture persisted without perceptible diminution for an interval as long as that occupied by the normal relaxation, and then subsided so slowly that it was difficult to fix the time of its completion. The following extracts from protocols illustrate this effect.

Cat. 3·6 kg. Right hypoglossal nerve cut under ether 20 days previously. Anæsthetized with chloroform and ether, followed by chloralose supplemented by occasional ether, as needed. Right chorda-lingual prepared. Mouth held open for inspection of the tongue, which falls into the roof of the mouth.

11.48. Stimulation of right chorda-lingual, coil at 12 cm., for 10 seconds. Maximal contracture of tongue. Relaxation begins promptly on cessation of stimulation, and takes about 7 seconds in completion.

11.54. Repeat same stimulation. Tongue reaches maximal contracture in a few seconds, and again takes about 7 seconds from cessation of stimulus for complete relaxation.

11.59. 0·5 mg. adrenaline into femoral vein.

12.0. Rise of arterial pressure just past the maximum. Stimulate right chorda-lingual, coil at 12 cm., for 10 seconds. Contracture complete in about 2 seconds. On stoppage of stimulation relaxation is delayed, proceeds very slowly when it begins, and is still incomplete at 1 *minute* after cessation of stimulus.

12.10. Repeat same stimulation. Contracture as before. Relaxation complete in 8 seconds.

The sequence was repeated with similar results.

Dog. 10 kg. Right hypoglossal cut under ether 15 days previously. Anæsthetized with ether followed by chloralose. Right chorda-lingual isolated. Arterial pressure from femoral artery, injections into femoral vein. Mouth held open for observation. Tongue lying flaccid in the roof of the mouth.

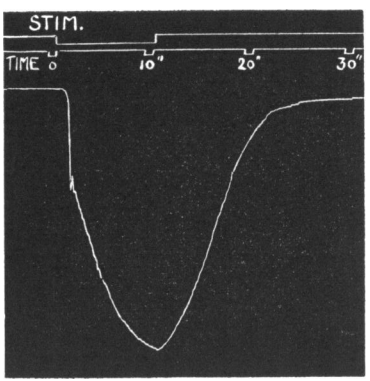
12.32. Stimulation of chorda-lingual, coil at 8 cm., for 10 seconds. Contracture, taking about 6 seconds to reach maximum, when tongue was raised to the level of the lifted lower jaw, drawn towards and curling round its right side. On stopping stimulation tongue begins to relax and has returned to original position in 13 seconds.

12.39. Repeat stimulation with similar result. Relaxation in 12 seconds.

12.43½. 0·5 mg. adrenaline intravenously.

12.44. Repeat chorda-lingual stimulation, coil at 8 cm., for 10 seconds. Contracture not perceptibly different in onset, rate, or extent from the previous ones. On stopping stimulation tongue did not begin to relax for 15 to 20 seconds, and then very slowly, the contracture not having disappeared completely after 1½ minutes.

12.54. Tongue now more sensitive to chorda-lingual stimulation. Stimulation for 10 seconds with coil at 14 cm. Contracture as before. Relaxation 11 seconds.



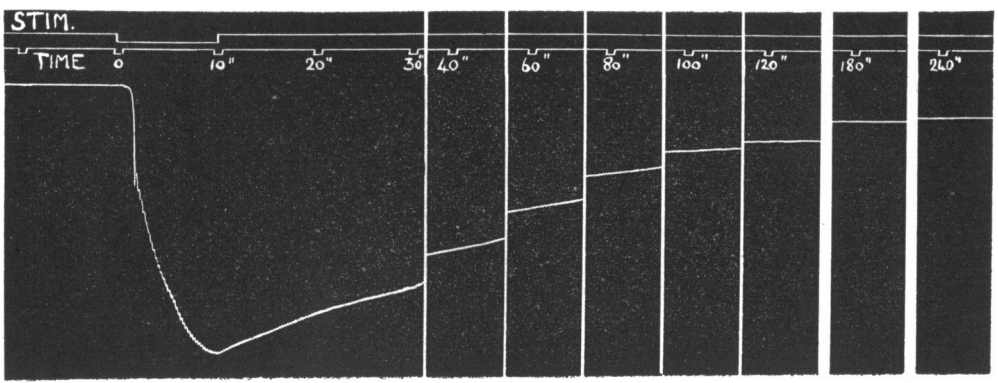


Fig. 4.

Fig. 5.

Fig. 4. Denervated tongue. Signal shows stimulation of chorda-lingual for 10 seconds.

Fig. 5. Same experiment as Fig. 4. Stimulation 1 minute after i.v. injection of 0·5 mg. adrenaline.

12.59. 1 mg. adrenaline intravenously.

1.0. Stimulation of chorda-lingual 10 seconds coil at 14 cm. Contracture as before. Relaxation just beginning 40 seconds after end of stimulation, and practically complete in 1 min. 40 sec.

The sequence was several times repeated.

We decided to observe more accurately the time relations of the effect, under normal conditions and with a large dose of adrenaline in circulation, by mechanically recording the movement of the tongue. Figs. 4 and 5, showing the records of the contracture in response to equal periods of nerve stimulation under the two conditions, show the characteristic after-prolongation of the contracture, produced by adrenaline. We observed such an essentially adjuvant effect of adrenaline in all our experiments on the Vulpian phenomenon save one. Thus our experience, with this single exception, confirmed that of the earlier observers who had detected no antagonism of adrenaline to this effect, and particularly that of Plattner and Reisch and of Orbeli and Fiedelholz, who had described it as having an adjuvant action. Plattner and Reisch found that adrenaline lowered the threshold of the Vulpian phenomenon in the cat, and Orbeli and Fiedelholz, to whose published results we have yet had access only in an abstract[1], observed lowering of the threshold, and increased extent and duration of response to a submaximal stimulation in the dog. The general run of our own experience was, therefore, in accordance with that of the majority of other observers. In one dog, however, we observed a depressant action of adrenaline on the Vulpian effect, and this was repeated with successive trials in this experiment. Fig. 6 shows this

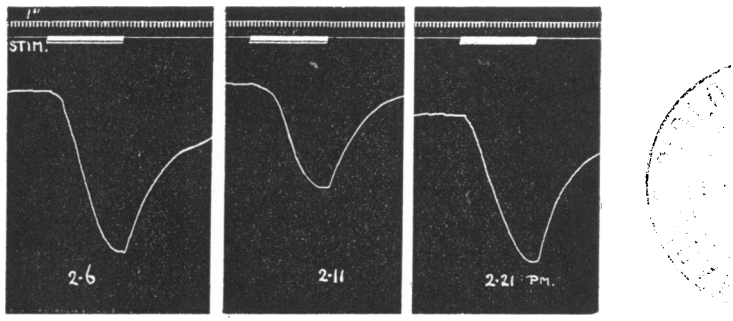


Fig. 6. Denervated tongue. Stimulation of chorda-lingual. 0·5 mg. of adrenaline i.v. at 2.10. Exceptional depressant action of adrenaline.

[1] We have had also a pleasant opportunity of seeing some of the tracings and discussing them with Prof. Orbeli, after our own had been obtained.

exceptional effect. The regular appearance of the phenomenon on repetition excluded the possibility of accident, such as short-circuiting of the electrodes. The effect was a real one, and we find no reason to doubt that the complete suppression of the Vulpian contracture by adrenaline described by Frank, Nothmann and Hirsch-Kauffmann who only record this one observation on the point, was a similarly genuine case of this exceptional effect of adrenaline.

We had indications, therefore, of two effects of adrenaline, in opposite directions, on the Vulpian phenomenon alone. In the case of the Sherrington phenomenon, which Hinsey and Gasser [1928] found to be suppressed by circulating adrenaline, our own observations frequently produced evidence of a depressant and an adjuvant effect, appearing as two phases of the action of one dose of adrenaline. We confirmed Hinsey and Gasser in finding that adrenaline depressed the contracture, at the height of its effect on the blood-pressure and for some time afterwards. In our experience, however, the depression never went so far as to abolish the response completely. When equal short periods of sciatic stimulation were given at intervals after the adrenaline injection, the maximum reduction of the response was reached at about 1 minute. Thereafter the contracture steadily recovered with successive stimulations and usually showed a phase of clear increase, both in strength and persistence, and finally returned to approximately the initial value. This after-phase of accentuation was as pronounced as the first phase of depression, but it tended, after several doses of adrenaline, to fade, so that depression became the only obvious effect of a later dose. Fig. 7 gives two records of the effect of adrenaline on the Sherrington phenomenon, both showing the primary depression, but only the upper one showing the subsequent adjuvant effect. The lowest record in this figure shows, for comparison, the complete extinction by adrenaline of the much stronger contracture evoked by a small arterial injection of acetylcholine. A similar but not quite complete suppression by adrenaline of the acetylcholine contracture of a denervated dog's tongue is shown in Fig. 8, the dose of adrenaline being such as habitually causes the increase of the corresponding nervous contracture shown in Figs. 4 and 5. To complete the account, we should mention the observation that adrenaline (0·1 to 0·2 mg.) injected into the carotid artery, so that it reaches the tongue in relatively high concentration, produces yet another type of effect on the Vulpian phenomenon. The response is initially but little altered, but shows a gradual reduction with successive stimulations. The depression does not reach its maximum until about 12

minutes from the injection, and then as slowly disappears. It seems to be directly related to the intense and prolonged ischæmia, which is

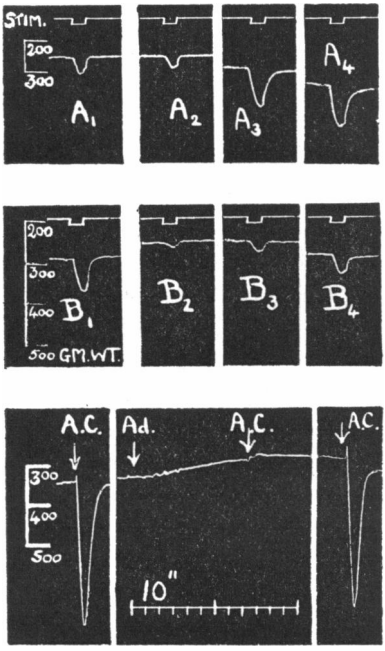


Fig. 7.

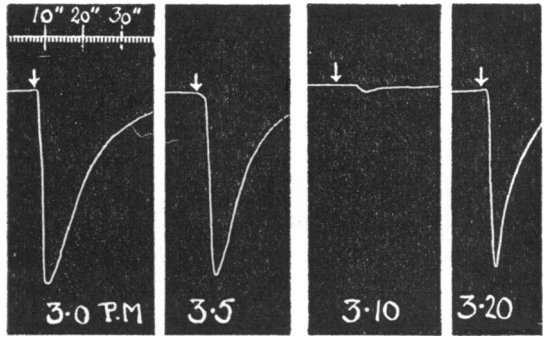


Fig. 8.

Fig. 7. Tension records of gastrocnemius (cat) denervated by root section. A, stimulations (antidromic) of dorsal root fibres in sciatic, in each case for 10 seconds, coil at 6 cm. B, same as A, later in experiment, coil at 5·5 cm. Between A_1 and A_2, and B_1 and B_2 1 mg. adrenaline i.v. At A.C. arterial injections of 10γ acetylcholine. At Ad. 0·2 mg. adrenaline i.v.

Fig. 8. Denervated tongue. Four arterial injections of 0·1 mg. acetylcholine. At 3.9, 0·5 mg. adrenaline was injected i.v.

visibly produced in the tongue by adrenaline given in this way. The same kind of gradual depression of the response follows stoppage of the blood flow by arterial clamping, and the pituitary vaso-constrictor principle ("vaso-pressin," 1 unit) produces a closely similar effect when injected in this way. It seems, therefore, that this adrenaline effect can be regarded as the result merely of vaso-constriction, producing a prolonged ischæmia. This was not the case with either of the effects on the contractures following general distribution of adrenaline by intravenous injections. These, whether of depression or augmentation, had come and gone before the depression due to complete ischæmia would have become

apparent. In their time relations they resembled rather the effect of adrenaline on the drug contractures; but, whereas intravenous adrenaline regularly and completely extinguished these latter, its effect on the contractures produced by nerve stimulation might be depression, augmentation, or both in succession.

(*b*) *Drug contractures.*

(i) *Artificially perfused muscle.* The contrast between the effects of adrenaline on nervous contractures and drug contractures respectively, though not so complete as a few experiments on the tongue alone would have suggested, was still substantial. In the hope of finding a clue to its meaning we made further experiments on the acetylcholine contracture under conditions of controlled circulation. For these experiments we used the denervated gastrocnemius of the dog, the leg being perfused with defibrinated dog's blood by the Dale-Schuster [1928] pump. Gaddum's [1929] flow recorder was used for continuous record of the venous outflow. Our aim was, by rapid adjustment of the throw of the perfusion pump, to compensate for the vaso-constrictor effect of adrenaline and thus prevent any reduction of the circulation rate. Small doses of adrenaline were used, injected into the arterial cannula, so that the effect was rapidly produced as the adrenaline passed through the vessels of the leg, and rapidly disappeared, as what remained of the small dose passed on to mingle with the general volume of blood in the apparatus. We were thus able to follow several times the phases of its effect on a following series of injections of acetylcholine, without producing any significant concentration of adrenaline in the blood as a whole.

Under such conditions the depressant effect of adrenaline on the action of acetylcholine is so brief as to be easily missed. If the acetylcholine injection follows that of adrenaline within 15 seconds, however, its action on the denervated muscle is completely suppressed; if an interval of 30 seconds has elapsed between the injections the contracture appears again; and at a minute or more from the time of the adrenaline injection, acetylcholine not only produces a contracture, but a definitely stronger and more persistent one than that evoked by the same dose before adrenaline was given. Fig. 9 shows these successive effects. We have, in fact, a sequence of effects which clearly recalls that produced on the Sherrington phenomenon by a larger dose of adrenaline in the general circulation—an initial depression followed by an accentuation and prolongation of the contracture, and then a gradual return to the normal response.

The possibility that the suppression of the drug contractures by adrenaline might be due to its vaso-constrictor effect, preventing passage

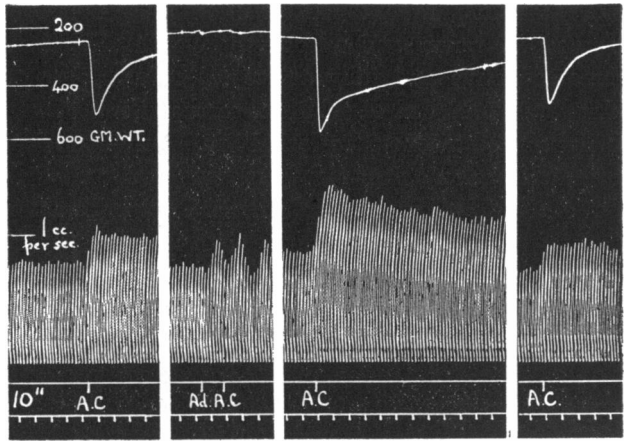


Fig. 9. Tension record of perfused denervated gastrocnemius (dog). Lower record—venous outflow. At A.C. and Ad., i.a. injections of 10γ acetylcholine, and 10γ adrenaline.

of the drug through the capillaries supplying the sensitive muscle, was discussed by earlier workers. Dale and Gasser produced evidence to show that the action was, at any rate, not wholly of this kind. The result above described, which could be regularly repeated, seemed to exclude it altogether. When the pump was adjusted to produce such a rise of arterial pressure that adrenaline did not even reduce the rate of blood flow, the acetylcholine contracture was still completely annulled. It was not possible to explain the undiminished blood flow by supposing that restriction of flow persisted in the muscles and was compensated by accelerated flow through the skin vessels. All the evidence points, on the contrary, to vaso-constriction by adrenaline involving the skin vessels more than those of the muscles. If adrenaline prevents the contracture by hindering access of acetylcholine to the sensitive muscle fibres, it must, therefore, do so by some action other than vaso-constriction. As to the relation of the subsequent accentuation of the drug contracture to the vascular conditions, the observed facts afforded no evidence. Clearly it was important to know whether, if the stimulating drug could be applied directly to sensitized mammalian muscle fibres, previous application of adrenaline would modify the resulting contracture, and if so in which direction.

(ii) *Isolated muscle.* For this purpose we used isolated strips from the diaphragm, denervated by section of one phrenic nerve under ether a fortnight previously. Our best preparations were obtained from young cats. The strip was cut parallel to the direction of the muscle fibres and, when suspended in warm and vigorously oxygenated Locke-Ringer solution, remained alive and fully active for many hours. The saline bath contained about 15 c.c. of solution, so that the addition of 2γ of acetylcholine produced a concentration of 1 in 7·5 millions. The reaction (Fig. 11) recorded here on a very slowly moving surface, is so slow as to resemble that of a strip of involuntary muscle. The maximum of shortening is not maintained, the curve beginning at once to fall towards a much lower level, from which, again, relaxation is accelerated by washing away the drug with fresh Ringer's solution. Other drugs of the class, such as tetramethylammonium salts (T.M.), produce slower and less rapidly evanescent, but otherwise similar contractures, but are active only in concentrations many times as great (Fig. 10). A control strip of muscle from the

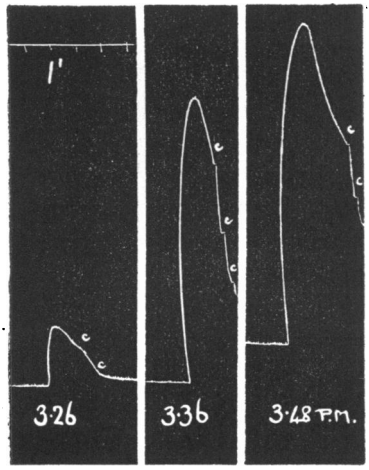


Fig. 10.

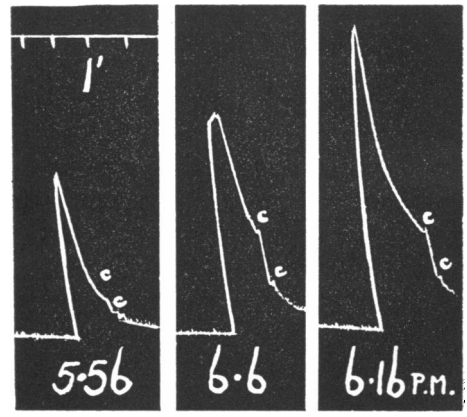


Fig. 11.

Fig. 10. Isolated slip of denervated diaphragm (cat). Isotonic record. Three applications of 0·25 mg. T.M. (1 in 60,000). At 3.28, 0·2 mg. adrenaline added to bath. Washed out at 3.38.

Fig. 11. Same as Fig. 10, later in experiment. Three applications of 2γ acetylcholine (1 in 7·5 millions). At 6.0, 0·2 mg. adrenaline added to bath. Washed out at 6.7.

normal, innervated half of such a diaphragm is quite indifferent to acetylcholine, in a dose many times that producing maximal contractures of the denervated strip. On such a preparation we could test the direct effect

of adrenaline on the drug contractures. A series of applications of the stimulant drug in a constant submaximal dose, given at intervals of about 10 minutes, produced a series of approximately equal contractures. 0·2 mg. of adrenaline added to the bath had no direct effect on the muscle, which remained fully relaxed. When, however, the dose of the stimulant drug used in the previous series was then added, a greatly enhanced contracture resulted, the degree of shortening and the persistence of the effect being both increased. The bath being then emptied, the preparation was washed by several changes and left in clean Ringer's solution. After the usual interval from the previous application, the same dose of the stimulant was again applied, and the resulting contracture showed a further enhancement. Only after several such applications and washings did the response return to the original dimensions. A further dose of adrenaline produced again an increased response, but the effect was less pronounced than at the first trial. Figs. 10 and 11 illustrate this effect of adrenaline on the responses to 250γ of tetramethylammonium iodide (T.M.) and 2γ of acetylcholine (A.C.) respectively. The increase of the contracture is more pronounced in the former case; but this is attributable to the fact that it was produced by a first application of adrenaline to the preparation, that with acetylcholine being obtained with a subsequent re-application. In both cases the further increase of the response, after the adrenaline has been washed away, can be clearly seen. We have made this experiment with preparations from several denervated diaphragms. In some cases the effect of adrenaline has been greater than in others; in some cases the increase of the persistence of the contracture has been more pronounced than increase of its amplitude; but the effect has in every instance been in the same direction.

It should be recalled in this connection that Riesser and Neuschloss [1921], Hess and Neergaard [1924], and Gasser and Dale [1926], all tried the effect of adrenaline, applied directly to isolated frog's muscles, on the contractures produced by acetylcholine similarly applied, and failed to detect any antagonism.

(*c*) *Discussion.*

With this direct action of the stimulant drugs to the denervated muscle we find no trace of the powerful antagonism, which adrenaline exhibits when they are administered by the circulation. This antagonism, accordingly, cannot be due to an action of adrenaline on the sensitiveness of the muscle itself to acetylcholine and its allies, but must be attributed

to its impeding at some point their passage from the circulating blood to the sensitive muscle fibres. We have already excluded vaso-constriction as an important factor of this hindrance, and the most likely alternative seems to be a reduced permeability of the capillary walls. Such a conception would account for the fact that circulating adrenaline does not at all weaken the action of acetylcholine on the blood vessels themselves; by producing a high arterial tone and thus giving scope for its relaxation, it may appear, indeed, to intensify the vaso-dilator action; at the same time it annuls the action of the drug on the denervated voluntary muscle fibres, to reach which it must pass through the capillary walls.

We have seen that the action of adrenaline on the contractures produced by nerve stimulation involves two opposite effects. In the case of the Vulpian phenomenon enhancement is the usual, depression the exceptional effect; with the Sherrington phenomenon depression and enhancement usually appear as successive phases in the action of one injection of adrenaline. Considering first the adjuvant action only, we find that this resembles the effect of adrenaline on the sensitiveness of the isolated muscle to acetylcholine and its allies, not only in its general direction, but even in detail. The increase of the contracture both in amplitude and persistence, its further enhancement as adrenaline is in process of removal from the muscle, and the weaker effect of later doses than of a first application of adrenaline—all these are easily traced in the effects of adrenaline on the drug contractures of the isolated muscle, and on the nervous contractures of the muscle with natural circulation. It would not be impossible to explain this parallel even on a non-humoral conception of the transmission to the muscle of the effect of the nervous impulses; we could suppose that adrenaline simply increases the sensitiveness of the muscle to any stimulus, whether chemical or nervous, which excites the contractures. In the effects of adrenaline on the nervous contractures we also find, however, the other depressant action, resembling that which it produces on the contractures elicited by injection of drugs. In the latter case we have found reason for attributing it to a hindrance of the access of the drugs to the muscle, and it seems quite impossible to explain its appearance in connection with the nervous contractures on any but a chemical theory of their production. If we adopt the conception, already suggested by other evidence, of the nervous contractures as due to the liberation outside the muscle fibres of a substance like acetylcholine, the curious complex of agreements and contrasts at once becomes intelligible. Such a substance, liberated at the

endings of the vaso-dilator nerve fibres on intramuscular arterioles, might reach the denervated voluntary muscle fibres by escaping directly into the intervening lymph, or by leaking into the blood and then secondarily diffusing through the capillary walls. In the former case adrenaline would increase the resulting contracture, in the latter case it would suppress it. If the stimulant substance normally reached the muscle in both ways, adrenaline might either diminish or increase the contracture, or produce both effects in succession. Such a conception provides a reasonable interpretation for an otherwise bewildering variety of effects; but it applies equally well to any substance sharing the dual action of acetylcholine, causing dilatation of normal arterioles and contracture of denervated voluntary muscle. We must look in another direction for evidence concerning the chemical nature of the substance actually concerned.

(3) *Action of other vasomotor agents.*

(*a*) *Sympathetic nerve stimulation.*

Ginezinski and Orbeli [1928] cut the hypoglossal nerve in the bony canal of its exit from the skull, before the addition to it of the sympathetic fibres from the superior cervical ganglion. After the usual interval for degeneration it was found that stimulation of the cervical sympathetic had a reinforcing effect on the contracture produced by chorda-lingual stimulation. The effect of sympathetic stimulation on the Vulpian phenomenon appears, therefore, to correspond to the usual effect of adrenaline on that response. We have ourselves, as yet, only tested the effect on the contracture of the cat's gastrocnemius evoked by arterial injection of acetylcholine. The gastrocnemius was denervated by aseptic section of the motor nerve roots under ether, and after 12 to 14 days the cat was again anæsthetized and made into a spinal preparation. The usual arrangements were made for recording the contracture of the gastrocnemius, and the sympathetic chain of the denervated side was isolated in the abdomen from the fourth to the sixth lumbar ganglia, cut anteriorly and laid on electrodes for stimulation. Control injections of acetylcholine were given so as to enter the iliac artery to the denervated leg, and the resulting contractures recorded. The sympathetic nerve was then stimulated faradically, and a minute after the beginning of the stimulation the injection of acetylcholine was repeated. The contracture was completely suppressed. Four minutes after the end of the period of sympathetic stimulation, a further injection of acetylcholine produced

again a normal contracture. Fig. 12 illustrates such a sequence, which was repeated at several trials in three different experiments. In one case

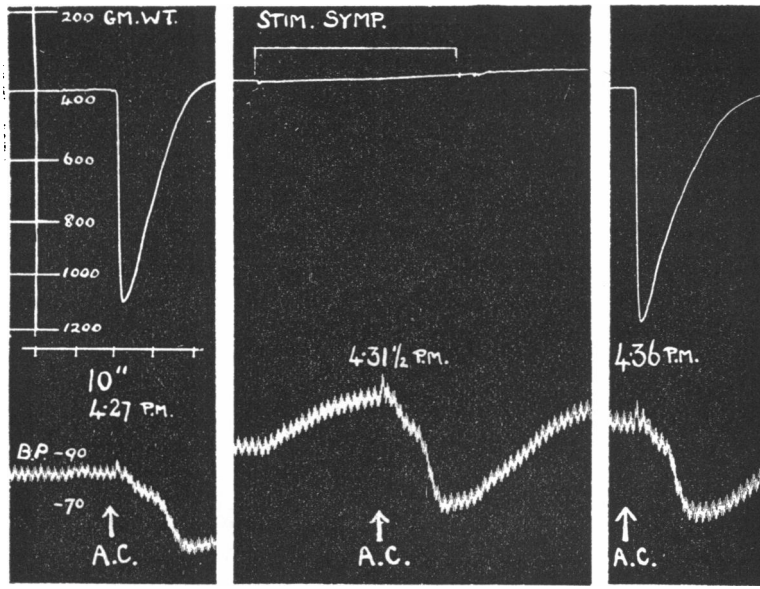


Fig. 12. Gastrocnemius (cat) denervated by root section. At A.C. three successive arterial injections of 1γ acetylcholine. Abolition of contracture by stimulating sympathetic supply to leg.

the second injection was made, not during continued sympathetic stimulation, but 20 seconds after its termination. A very small contracture was produced, and it was evident that the antagonistic effect of the stimulation long outlasted the actual passage of nerve impulses.

So far as evidence is available, therefore, it would appear that the effects of sympathetic stimulation on the nervous and drug contractures closely resemble those of adrenaline in either direction. It would be of special interest to know whether sympathetic stimulation would show also a depression of the Sherrington phenomenon, but we have not yet made the experiment.

(*b*) *The pituitary pressor principle.*

Gasser and Dale [1926] tried the effect of strongly vaso-constrictor doses of barium chloride on the contracture produced by acetylcholine in the cat's denervated gastrocnemius, with natural circulation. They observed no significant change in the response, and used the fact as an

argument against the attribution of the adrenaline antagonism to its vaso-constrictor action. We have here given additional evidence in support of that conclusion, and have suggested a reduction of capillary permeability as a more likely explanation of the effect. It seemed desirable, accordingly, to examine the action in this direction of the pituitary vaso-constrictor principle (vaso-pressin) to which Krogh [1922] has attributed an important action in reducing the permeability of capillaries in the frog. We found that it had, indeed, a depressant action on the contracture response to arterial injections of acetylcholine, similar to that produced by adrenaline. It was slower in onset and in disappearance than the adrenaline effect, and did not so regularly cause a complete suppression of the acetylcholine contracture at any stage of its action. In some instances, however, its antagonistic action was, at its maximum, apparently as effective as that of adrenaline. Fig. 13 illustrates such an

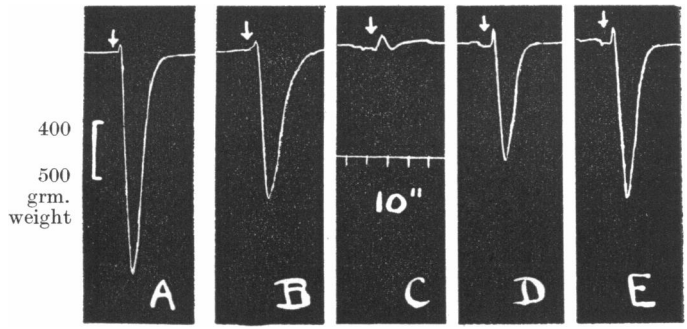


Fig. 13. Denervated gastrocnemius. Arterial injections of 10γ acetylcholine. A, normal effect. B, C, D and E, effects $2\frac{1}{2}$, 5, 10 and $13\frac{1}{2}$ minutes after 1 unit of vaso-pressin i.v.

effect on the acetylcholine contracture of a cat's denervated gastrocnemius. Of the nervous contractures we only tested the effect of vaso-pressin on the Vulpian phenomenon, which was not clearly weakened or accentuated by this hormone. In conformity with this observation was one made on its application to the isolated strip of denervated diaphragm, in which it caused no definite change of response to acetylcholine. Its one clear effect, therefore, was on the contractures produced by acetylcholine injected into the blood stream. These it reduced or suppressed; so that its action was in accordance with the other evidence as to its effect on capillary permeability, and with our own interpretation of the similar action of adrenaline.

(c) *Histamine.*

If we were right in attributing the depressant actions of adrenaline and vaso-pressin, on the drug contractures *in vivo*, to lowered permeability of the capillary walls, it seemed probable that histamine, which increases the permeability, and has been regarded as an antagonist of adrenaline in that respect, would act in the opposite direction on the production of the contracture. To test this possibility we recorded the contractures in response to equal, small, arterial injections of acetylcholine, in the denervated gastrocnemius of a spinal cat. A slow, steady intravenous infusion of adrenaline was given, at a rate sufficient to reduce the acetylcholine contractures to small dimensions, and 0·5 mg. of histamine was then injected intravenously, the adrenaline infusion being steadily maintained. One minute later, when the action of the histamine, as judged by the fall of arterial pressure, had about reached its maximum, acetylcholine produced a contracture as strong as those before adrenaline, and more prolonged. Five minutes later the acetylcholine contractures were again subject to the reduction due to adrenaline alone.

In another experiment, also on the denervated gastrocnemius of a spinal cat, 0·5 mg. of histamine and 0·2 mg. of adrenaline, injected together into a vein, caused only a reduction of an acetylcholine contracture, which was completely suppressed by 0·2 mg. of adrenaline alone.

These results support our view concerning the nature of the adrenaline action. Mention should be made, however, of one experiment in which, without adrenaline, we tried the effect, on the contracture produced by a given dose of acetylcholine, of injecting 0·2 mg. of histamine in advance of it into the same artery. The result was a definite reduction of the contracture. Such a dose of histamine, so injected, would certainly produce constriction of the arterioles. It can be shown, indeed, in an experiment with artificial perfusion, that mere reduction of the rate of blood flow through a muscle will reduce the effect of a small dose of acetylcholine. It is quite clear, however, that this plays an insignificant part in the antagonism of adrenaline to the contractures.

(4) *Action of eserine.*

By further study of the influence of eserine on the production of contractures in denervated muscles, we hoped to obtain some indication of the chemical nature of the substance peripherally liberated, and responsible for the nervous contractures. There was already a large body

of evidence showing that the effects of small doses of eserine, in contrast to those of pilocarpine, on the activity of organs receiving autonomic innervation, are due to its increasing the effectiveness of parasympathetic nerve impulses [cf. Anderson, 1905; Winterberg, 1907; Prevost and Saloz, 1909; Loewi and Mansfeld, 1910; Dixon and Ransom, 1912; Heinekamp, 1925; Gibbs, 1926]. On the effects of true sympathetic nerves it has no such effect [Heinekamp, 1925; Granberg, 1925]. Loewi [1912] had shown, moreover, that the effects on the rabbit's blood-pressure of pilocarpine and muscarine, though they so closely follow the actions of parasympathetic nerves, are not increased by eserine. Reid Hunt [1918], on the other hand, had shown that both vascular effects of acetylcholine—the "muscarine-like" action normally produced by small doses, and the "nicotine-like" action of larger doses given after atropine [Dale, 1914]—were intensified by eserine. He had earlier [1915] found that the parasympathetic effects of homologues of acetylcholine were similarly increased by eserine.

Other evidence has definitely related the enhancement by eserine of the effects of acetylcholine to the esteric structure of the latter. Führer [1918] found that soaking the plain muscle of a leech in a very weak solution of eserine (1 in 1 million) did not alter the stimulant action on it of pilocarpine or of choline, but increased that of acetylcholine a millionfold. He suggested that the effect of eserine was to inhibit hydrolysis of acetylcholine by the tissue. Loewi and Navratil [1926], finding that eserine prolonged the inhibitory effect of vagus stimulation on the frog's heart, and likewise that of the inhibitory substance ("vagus substance") liberated by vagus stimulation and that of acetylcholine, but not that of muscarine or choline, investigated the action of eserine on the ferment in the frog's heart muscle which hydrolyses both the vagus substance and acetylcholine, and thereby destroys their activity. In both cases eserine inhibited this destruction. Later Engelhart and Loewi [1930] have found that eserine similarly inhibits, in very high dilutions, the rapid destruction of acetylcholine by normal blood investigated by Galehr and Plattner [1927]. Dr K. Matthes, who has been independently investigating in this laboratory the inhibition by eserine of the destruction of acetylcholine in the blood, has obtained results similar to those of Engelhart and Loewi and has extended the enquiry in several directions. Among his observations, which are in course of publication, are some on the synthetic compound "miotine" prepared by Dr Stedman of Edinburgh, and representing a part of the eserine molecule. Miotine had been shown to resemble eserine closely

in physiological action, and Matthes has found that it equally inhibits the destruction of acetylcholine by the blood esterase.

These various items of evidence, connecting the physiological effects of eserine with inhibition of an esterase action, gave great additional significance to its effects on the excitation, by different methods, of contractures of denervated muscles. It was already known [Nitschke, 1923; Dale and Gasser, 1926] that a small dose of eserine greatly lowered the threshold dose of acetylcholine for the production of a contracture of the denervated cat's gastrocnemius. Eserine, however, apart from recent evidence concerning the mechanism of its action, has long been reputed to have a general effect in increasing the excitability, even of normal muscle, to any kind of stimulus. It was important, therefore, to be sure that, in the range of dosage with which we were concerned, we were not dealing merely with such an unspecific effect. We needed, as a control, a non-esteric substance having, on denervated muscle, a stimulant action of the same type as that produced by acetylcholine. We chose tetramethylammonium iodide (T.M.), as being a substance closely allied in its chemical nature to choline, relatively powerful as an excitant of contractures in the denervated muscle, and producing a practically equal series of these when given in a series of suitable, equal doses. We have tested the effects of T.M. in comparison with those of acetylcholine, before and after the administration of a small dose of eserine, on the denervated dog's tongue and cat's gastrocnemius, with natural circulation and arterial injections, and on the isolated slip of denervated cat's diaphragm. The results were all in the

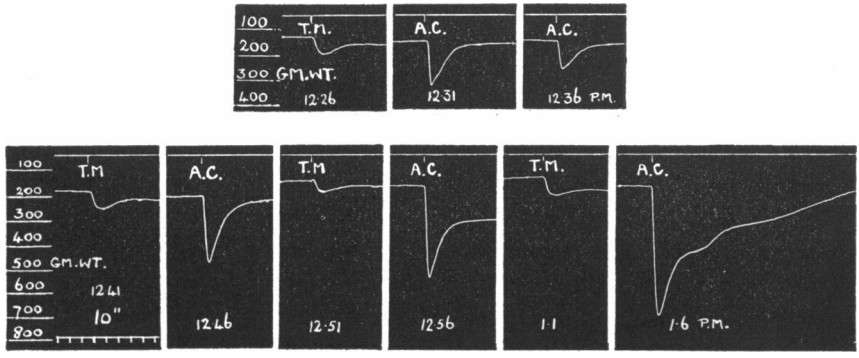


Fig. 14. Denervated gastrocnemius. Contractures in response to arterial injections of T.M. (10γ) and acetylcholine (A.C.) 0·2γ before and after eserine. At 12.36½, 0·1 mg. eserine i.v.

same direction, and those on the cat's gastrocnemius and the isolated diaphragm are shown in Figs. 14 and 15. In every case the effect of a submaximal dose of acetylcholine was greatly increased after eserine, while that of a submaximal dose of T.M. was not perceptibly altered. The fact, evident in Fig. 14, that the increase of sensitiveness to acetylcholine, caused by eserine, took some time for its full development, had an interesting parallel in the similarly slow development of the inhibitory action of eserine on the hydrolysis of acetylcholine in blood. It seemed reasonable, accordingly, to conclude that the action of eserine, in increasing the contracture effects of acetylcholine while leaving unchanged those of the non-esteric T.M., was due to its antagonism to the action of a ferment which hydrolyses choline esters.

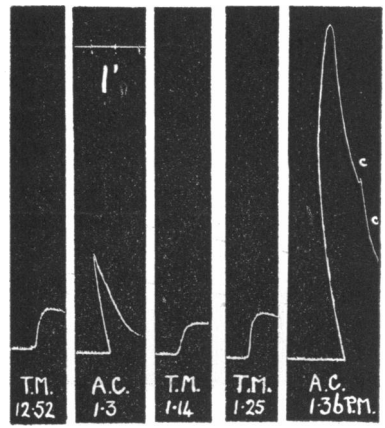


Fig. 15. Isolated strip of denervated diaphragm (cat). Applications of T.M. (0·2 mg.) and A.C. (2γ) before and after eserine. 0·015 mg. eserine (1 in 1 million) added to bath at 1.17, washed out at 1.26½.

In the light of this conclusion, the effects of similar doses of eserine on the contractures produced by nerve stimulation acquired a special

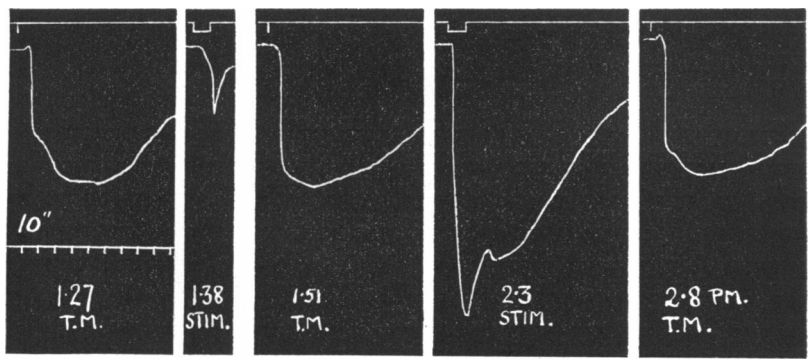


Fig. 16. Denervated tongue. Effects of stimulating chorda-lingual for 10 seconds (coil at 10 cm.) compared with those of arterial injections of T.M. (0·3 mg.) before and after eserine. 0·1 mg. eserine i.v. at 1.41.

significance. Fig. 16 shows that eserine caused a great increase of the contracture of the denervated tongue in response to chorda-lingual

stimulation, while it again failed to produce any significant change in the response to a submaximal injection of T.M., used as a control. Fig. 17 shows the similar effect of "miotine" on the Sherrington

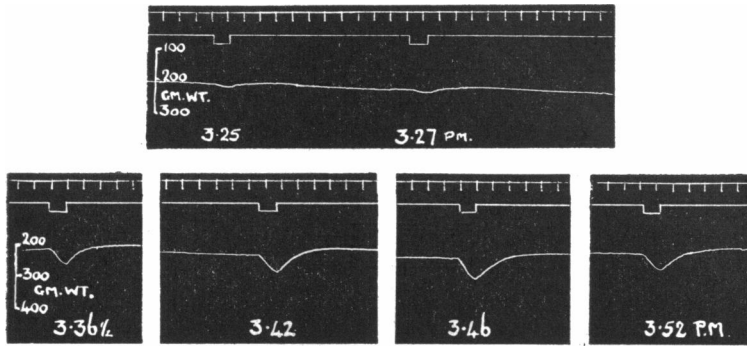


Fig. 17. Gastrocnemius (cat) denervated by root section. Responses to stimulation of sciatic for 10 seconds (coil at 5 cm.). At 3.30, 0·1 mg. and at 3.43½, 0·2 mg. of "miotine" i.v.

phenomenon. As the result of several earlier periods of stimulation of the sciatic nerve the mechanism had become so far exhausted that further stimulation caused a contracture of only minimal tension; after 0·1 mg. of miotine, the contracture acquired, with successive periods of stimulation, a strength greater than that which it had shown originally in the unfatigued preparation, and another injection of 0·2 mg. of miotine caused a further increase.

We have discussed above the evidence, especially from the effects of adrenaline, which seems to us decisive for the production of the nervous contractures by peripheral liberation of a chemical stimulant. This must belong to the group of substances which cause dilatation of normal arterioles and contracture of motor-denervated voluntary muscle. The effects of eserine further limit our choice, among the known substances having this dual action, to a choline ester readily hydrolysed by the tissues. Acetylcholine, the only choline ester which has been shown to exist in the animal body, is pre-eminent in physiological activity in both the required directions, and in its liability to the hydrolytic destruction which eserine specifically inhibits.

(5) *Action of atropine, etc.*

We have, as yet, made only few and scattered experiments with the alkaloids of this group, but the results seem worthy of record, as they

tend to remove certain recorded anomalies of their action in relation to the contractures produced by acetylcholine. It is known, for example, that atropine has a definite antagonism to the production by acetylcholine of contractures in certain normal muscles of the frog [Riesser and Neuschloss, 1921]. The proportion of atropine required is higher than that which will suppress the parasympathetic effects of acetylcholine, but the antagonism is clear. In the mammal, on the other hand, no antagonism to the contractures of denervated muscle, produced by injection of even minute doses of acetylcholine, has been observed with doses of atropine many times as great as those which abolish its parasympathetic effects. Frank, Nothmann and Hirsch-Kauffmann [1923] state that scopolamine, in a dose of 15 mg., will abolish the contractures. We have injected scopolamine in the dose employed by these authors without obtaining even a significant reduction of the contractures of denervated muscles.

On this evidence a case could have been made for classing the contractures excited in certain normal frog's muscles with the parasympathetic effects of acetylcholine, and Frank and his colleagues had, indeed, based on these effects a suggestion that these muscles of the frog have probably an additional, parasympathetic nerve supply. The action of acetylcholine on denervated mammalian muscle, on the other hand, quite clearly belongs to the other, nicotine-like, aspect of its action. Since the experiments on denervated mammalian muscle had all been made by injecting atropine and acetylcholine into the circulating blood, while in those on the frog's muscle the drugs had been directly applied to the isolated tissue, it seemed worth while to test their effects on the isolated strip of denervated diaphragm. In our few experiments on this point we have used rather high concentrations of atropine, but their complete suppression of the response to a previously effective dose of acetylcholine revealed a real antagonism, of which experiments made by intravascular injection had given no hint. Fig. 18 shows the suppression by 5 mg. of atropine, added to the bath containing 15 c.c., of the effect of 5γ of acetylcholine, and the restoration of the response with subsequent washing of the preparation. Atropine in this dosage (1 in 3000) similarly annulled the action of previously effective doses of T.M. In the lower dose of 0·1 mg. (1 in 150,000) atropine still caused a pronounced reduction of the effect of 2γ of acetylcholine (Fig. 19); and in this case we are dealing with an atropine concentration of the same order as that which appears to be quite ineffective, when both drugs are given by intravascular injection in the living animal.

We have not attempted to determine the lowest effective dose of atropine, with direct application, in relation to a particular dose of

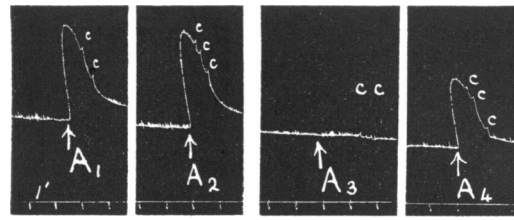


Fig. 18. Isolated strip of denervated diaphragm (cat). Four successive applications of 5γ acetylcholine. 6 minutes before A_3, 5 mg. atropine sulph. added to bath (*i.e.* 1 in 3000). c, change to clean Ringer's solution.

acetylcholine. The results obtained, however, are sufficient to show that the reaction of denervated mammalian muscle presents no sharp contrast to normal frog's muscle, with regard to this particular antagonism.

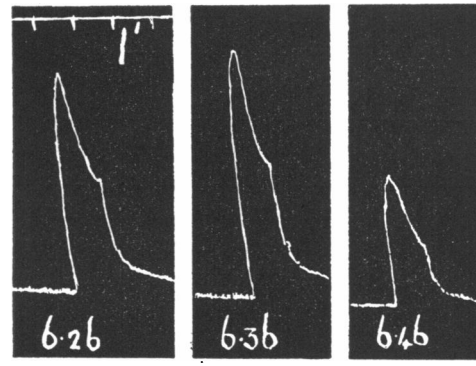


Fig. 19. Similar to Fig. 18. Three successive applications of 2γ acetylcholine. At 6.38, 0·1 mg. atropine sulph. added to bath (*i.e.* 1 in 150,000).

Atropine is a less potent antagonist of acetylcholine in the former case, but the difference is only quantitative. There is no more reason for assuming a direct parasympathetic innervation of the voluntary muscle fibres in the one case than in the other.

IV. General discussion.

In the sections dealing with the different groups of our experiments we have already considered the significance of the individual results in some detail. Here we need only review their general effect and consider

certain points which do not arise so directly from them. Our aim was to test the hypothesis attributing the normal vaso-dilator effects of impulses in parasympathetic and dorsal-root nerve fibres to peripheral liberation of acetylcholine, and the contractures of motor-denervated muscles, excited by stimulating such nerves, to the leakage of this substance on to the muscle fibres rendered sensitive to it. We met at the outset certain effects of adrenaline which seemed to be incompatible with such a conception. A closer investigation resolved the apparent discrepancy, and replaced it with new points of concordance between the nervous and chemical contractures. The effects of eserine limited the choice, among known substances producing both vaso-dilatation and contractures of denervated muscle, to the choline esters. We have still to consider one outstanding difficulty, which has been urged against the identification of this substance as acetylcholine, namely, the fact that the vaso-dilator effects of acetylcholine are readily and completely suppressed by doses of atropine which leave those of the nerves practically intact. We must consider the full consequences of attributing a decisive significance to this particular contrast in the action of atropine.

We may note, in the first place, that atropine paralyses the vaso-dilator actions, not only of acetylcholine, but of all the known choline esters; the liability to this antagonism is, indeed, characteristic of the action of choline itself. Those who adopt the humoral theory of transmission, therefore, and accept the evidence of the atropine action as deciding the nature of the transmitter, must regard choline esters as excluded wherever atropine fails. That is to say, they must reject the evidence afforded by the action of eserine, in favour of that afforded by atropine. A new set of difficulties then presents itself. The one case of a parasympathetic effect, in which the process of humoral transmission has been made accessible to direct investigation, is that of inhibition of the frog's heart by the vagus. Loewi and his co-workers demonstrated the liberation of the substance and showed that it had all the properties of an unstable ester of choline. They further showed [Loewi and Navratil, 1924] that atropine paralysed the vagus effect, not by preventing the liberation of the substance, but by preventing its action when liberated. Brinkman and Van Dam [1922] showed that the substance liberated by the heart vagus, not only inhibited the activity of the heart muscle, but stimulated that of the stomach. So far the story is clear. The transmitter is, in this case, a choline ester, having all the properties of acetylcholine; atropine paralyses its action, and accordingly that of the nerve impulses which liberate it. When, therefore, we find

that atropine paralyses only partially, or not at all, the effects of the vagus on the different parts of the gastro-intestinal canal, we have, if we accept the action of atropine as decisive in this matter, only two choices. We may suppose that vagus effects not paralysed by atropine are not humorally transmitted at all, or that the transmitter is not a choline ester; but in the latter case, we shall have to postulate not one, but several other transmitters of vagus effects, with different degrees of liability to the antagonism of atropine.

The actions of the chorda tympani have a special interest for our purpose. Atropine discriminates sharply between its stimulating effect on the secretory cells of a salivary gland, and its dilator effect on the arterioles, readily and completely paralysing the former, but hardly affecting the latter. We must either suppose then, that the effects on the gland cells is humorally transmitted while the vaso-dilator action is not, or that the transmitters are different substances in the two cases. In the case of the actions of the chorda on the denervated tongue the difficulties become even more obvious. If we reject humoral transmission of the vaso-dilator effect, because this is resistant to atropine, we must reject all the evidence, including that here produced, which indicates humoral excitation of the contracture of the denervated muscle. If, with Gasser, we accept humoral transmission of both effects, but exclude a choline ester as the agent, we reject the evidence of eserine, in order to accept that of atropine.

The assumption that the effect of atropine is thus decisive evidently does not remove all difficulties; it rather creates new ones. We are the more disposed to doubt its validity, by our own evidence as to the dependence of certain drug antagonisms, including one involving atropine itself, on the method of application. We know very little as to the nature of nerve endings in the plain muscle of different organs, or as to their relation to the surface membranes of the fibres; so that, even on a chemical theory attributing the action to liberation of a choline ester at the nerve terminations, we should have no right to regard its method of access to the receptive mechanism in the muscle fibre as everywhere identical with that of a choline ester injected into the blood stream. In the case of one parasympathetic action, indeed, that on the frog's heart, we have good reason for assuming such identity; and in that case atropine paralyses nervous and chemical inhibitions alike. Where, in other cases, it still suppresses the effects of choline esters artificially applied, but fails to paralyse the similar nervous effects, it is still possible that the latter might be due to the liberation of a choline ester, but in a

relation of so much greater intimacy with the receptive mechanism that atropine cannot prevent its access thereto. We know nothing of the mechanism of the atropine paralysis, but for purely diagrammatic purposes we may regard it as creating a barrier, which a choline ester cannot pass. If such an ester is liberated at the parasympathetic nerve endings, to act as transmitter of the effects of the nerve impulses, the latter will be paralysed completely, or partially, or not at all by atropine, according as the liberation takes place wholly without, partially within, or wholly within the barrier. There is no method yet obvious of putting this possibility to the test of experiment; but it includes all the facts here under consideration in one relatively simple conception, and does not entail the rejection of one line of evidence in favour of another.

In addition to the contractures of motor-denervated muscle evoked by parasympathetic or antidromic sensory nerve stimulation, there is one case in which stimulation of a true sympathetic nerve apparently produces a similar effect. According to Rogowicz [1885], the muscles moving the lips in the dog acquire a new power of contracting in response to stimulation of the cervical sympathetic nerve, when their normal motor supply through the facial nerve is caused to degenerate. We have not made experiments on this phenomenon ourselves. It is natural to associate it with the fact, observed by Dastre and Morat [1880] and often since confirmed, that the vessels of the bucco-facial area in the dog usually react to sympathetic stimulation by dilatation, in contrast to the constrictor reaction in all other superficial areas. It is certain that stimulation of the sympathetic nerve supply does not cause contracture in most denervated muscles; in the denervated muscle of the cat's leg we have seen that its effect is to suppress the response to acetylcholine, as adrenaline also does. The Rogowicz effect might, conceivably, be due to the presence in the cervical sympathetic of small dorsal root fibres of the type causing vaso-dilatation. There is another possibility, namely, that certain fibres, in the cervical sympathetic nerve of species showing this effect, though belonging anatomically to the true sympathetic system, may resemble in their action, and in the liberation of a choline ester as the transmitter of this, the fibres of the parasympathetic system. There are sympathetic fibres elsewhere which show such an exceptional pharmacodynamic relationship; the action of those exciting secretion in the sweat glands is as readily paralysed by atropine as that of the chorda tympani on the salivary gland, and in general the action resembles that of pilocarpine and acetylcholine on sweat secretion rather

than that of adrenaline. A further study of the Rogowicz phenomenon is clearly desirable.

It is obvious that the suggestion of a humoral transmission for certain effects produced by parasympathetic nerves can logically be applied to all their effects, which are chiefly on plain muscle. The variations, here described, in the delay of onset and rate of development of the contractures of the denervated tongue, with variations in the rate of application of shocks to the chorda-lingual nerve, show a general similarity to those recorded in the response of the plain muscle of an organ like the stomach to varied spacing of shocks applied to its motor nerve [McSwiney and Robson, 1929]. The denervated voluntary muscle, indeed, behaves in response either to the nerve impulses or to direct chemical stimulation in many respects like plain muscle; and there are features in the response of either to nerve stimulation which recall the phenomena of "recruitment" and "after-discharge" described by Sherrington in the behaviour of a reflex spinal centre to sensory impulses. In all these cases the accumulation of the effects of individual impulses suggests the intervention of a chemical agent. Only, however, in the cases of the effects of the vagus on the heart, as studied by Loewi and his co-workers, and of the contractures elicited by stimulating certain vaso-dilator nerves, does the evidence give a specific indication of the chemical nature of the substance concerned. Adoption of a chemical theory of the transmission of parasympathetic impulses further involves recognition of a strong probability that some analogous process intervenes in transmitting the very similar effects of impulses in true sympathetic nerves to the effector cells. In this case the existence of a true hormone adrenaline, so closely duplicating the effects of sympathetic nerves in its action, long ago gave rise to the suggestion, originally advanced by Elliott [1904], that liberation of this substance occurred at the endings of sympathetic nerve fibres. There is, again, in this case evidence of an accelerator substance liberated in the frog's heart by stimulating the sympathetic nerve supply [Loewi, 1921]. The direct evidence of chemical transmission is, however, less clear for the sympathetic than for the parasympathetic nerves, and the only hint as to the chemical nature of the transmitter is provided by the physiological similarity to the action of adrenaline. As the transmitter of parasympathetic effects, we consider that the evidence now available, including that here presented, makes a very strong case for acetylcholine.

We have, in agreement with Hinsey and Gasser, and with the support of our own observations, grouped with the proper parasympa-

thetic nerves, as regards the pharmacodynamic transmission of their arterio-dilator action and the associated contracture of denervated voluntary muscle, the dorsal root fibres responsible for the so-called "antidromic" vaso-dilator action. Here again the pharmacodynamic classification appears to cut across the anatomical; but if acetylcholine transmission is accepted in the one case, we consider that it must apply to both. We should make it clear, however, that this conclusion does not at all conflict with that of Lewis and Marvin [1928], who deduced from their experiments a liberation of *H*-substance, analogous in action to, if not identical with histamine, at the *sensory* endings, in the skin, of the dorsal root fibres, when they were antidromically stimulated. We are concerned with the other end of the terminal axon branching—with the endings on the arteries of the arterio-dilator collateral. Whether this is activated by antidromic impulses produced by artificial stimulation of the main sensory fibres, or by an axon reflex caused by irritation of the sensory endings, we suggest that the resulting arterial dilatation, corresponding to Lewis's reflex "flare," is produced, like the parasympathetic vaso-dilatation, by liberation of acetylcholine in relation to the plain muscle of the arterioles.

V. Summary.

1. The apparent difference between the actions of adrenaline on "drug contractures" and "nervous contractures" of denervated muscles has been investigated and explained.

2. Eserine greatly enhances the contractures produced by stimulating vaso-dilator nerves and those produced by choline esters, but leaves unchanged those produced by analogous non-esteric substances (tetramethyl ammonium salts).

3. Atropine paralyses the effect of acetylcholine and similar drugs, applied directly to denervated mammalian muscle.

4. The evidence removes discrepancies and produces new concordances, supporting the view that the vaso-dilator effects of parasympathetic nerves and of sensory fibres stimulated antidromically, and the contractures of denervated muscles accompanying these actions, are due to the peripheral liberation of acetylcholine.

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